RDM Division of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment

		DATE:	21st Aug 2017
DEPARTMENT: Cardiovascular Medicine WTCHG **LOCATION OF WORK:** Room 10/088 (Lab 3)	**PERSONS INVOLVED:** Watkins Group See attached sheet	**OTHERS AT RISK:** Others in Lab 3 only if contamination of other lab surfaces occurs and is not detected by monitoring	

DESCRIPTION OF PROCEDURE:

AMP Kinase Assays using ^{32}P – see attached protocols

SUBSTANCES USED	QUANTITIES USED	FREQUENCY OF USE	HAZARDS IDENTIFIED	EXPOSURE ROUTE	DOSE PER PROCEDURE (µSv)
[γ-^{32}P]ATP 220TBq/mmol 370MBq/ml 6000Ci/mmol 10mCi/ml	Working solution made up using 3.7MBq ^{32}P taken from a stock pot containing max 9.25 MBq. 0.0185 MBq per sample and maximum of 24 tubes per assay so 0.444MBq per assay.	Approximately twice per month	High energy β- emitter (1.71MeV). Skin dose rate from 1MBq point source at 30cm is 0.12mSv/h [Delacroix et al. *Radiat. Prot. Dosim.* **98**, 2002]	Mainly skin contact (mostly fingers). Some aerosol risk in this procedure.	*Whole body:* Effectively zero *Extremities:* See dose calculation below

COULD A LESS HAZARDOUS SUBSTANCE (OR FORM OF THE SUBSTANCE) BE USED INSTEAD? No

COULD A LOWER ACTIVITY BE USED? No
JUSTIFY QUANTITY OF MATERIAL IN USE: Quantities used are optimised to be the minimum necessary.

WHAT MEASURES HAVE YOU TAKEN TO CONTROL RISK?

ENGINEERING CONTROLS:
Perspex screen and radiation protection cabinet.
Stock pot shipped and stored encased in lead.
Tubes kept in Perspex racks with lids.
Work undertaken in fume hood where appropriate used to minimise aerosol escape.
Reduce dosage by control of distance, time and shielding.

PPE:
Lab coat, nitrile gloves and safety eyewear

MANAGEMENT MEASURES:
Follow the As Low As Reasonably Practicable (ALARP) rule
Adhere to local rules, EPR2010 and IRR99
Monitor work area before, during and after use
Lone working prohibited
Work only in designated Supervised areas
Radioactivity stock pot stored in locked refrigerator in Room 10/088
Adhere to limits of designated sink and bins
Adhere to storage time limits for bins [12 months for both 'solids' green/yellow band bin & 'organic liquids' orange bin]

CHECKS ON CONTROL MEASURES:
Regular monitoring and supervision
Checks on documentation – control of Monthly returns

Radiation monitor: **EP15**	**TRAINING REQUIREMENTS:** URPO lecture "Working with unsealed radioactive sources" and in-house training.
Is dosimetry required? No	

EMERGENCY PROCEDURES:
Refer to Contingency Plans in Local Rules & University Policy Statement S8/05: Appendix 16.
Decontamination where necessary with Count-Off and/or Decon90 decontaminants as recommended. Dispose of contaminated materials in designated bins and sinks. For any incident beyond minor contamination of radioactive workspaces alert RPS (and SRPS/URPO if appropriate).

WASTE DISPOSAL:

Aqueous	~79.4% to designated sink
Solid	~20% to green-yellow band bin
Organic liquid	~0.6% to orange bin

Are overall risk control measures adequate?	Yes	
NAME OF RADIATION PROTECTION SUPERVISOR:	Dr James Brown	**SIGNATURE**: 

Date of routine review	**DATE:**	/ / 2018	/ / 2019	/ / 2020	/ / 2021	/ / 2022
	BY:					

RDM Division of Cardiovascular Medicine
University of Oxford

Radiation Risk Assessment Training Record

AMP Kinase Assays using ^{32}P

The undersigned have read the above radiation risk assessment and understand the safety arrangements required and their own obligations in ensuring compliance.

DATE	NAME & SIGNATURE	TRAINED BY (NAME & SIGNATURE)

DOSE ESTIMATION

D. DELACROIX, J.P. GUERRE, P. LEBLANC AND C. HICKMAN

Phosphorus - 32

$^{32}P_{15}$

Half life: 14.3 days
Specific activity: 1.06E+16 Bq.g^{-1}

Risk group: 2
Risk colour: Orange

Main emissions (keV)

	Gamma or X		Beta (Emax)		Electrons		Alpha	
	E	%	E	%	E	%	E	%
E1			1710	100				
E2								
E3								
% omitted				0				

Exemption levels	
Quantity (Bq)	1E+05
Concentration (Bq.g^{-1})	1E+03

Transport (TBq)	
IAEA ST1 A_1 value	5E-1
IAEA ST1 A_2 value	5E-1

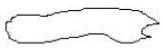
EXTERNAL EXPOSURE (mSv.h^{-1}) for an activity of 1 MBq or 1 MBq.m^{-2} (as appropriate)

Point source (30 cm)

Betas, electrons (skin dose): 1.18E-1

Gammas, X rays (deep tissue dose): 0.00E+0

Infinite plane source



Betas, electrons (skin)

10 cm	1.4E-01
1 m	4.8E-02

Photons (skin)

10 cm	0.0E+00
1 m	0.0E+00

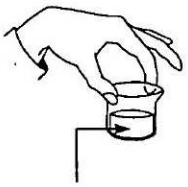
Photons (deep dose)

10 cm	0.0E+00
1 m	0.0E+00

10 ml glass vial

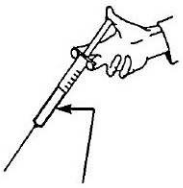
100 cm: 1.31E-6

Contact with 50 ml glass beaker



7.11E-4

Contact with 5 ml plastic syringe



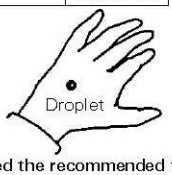
2.39E+1

The values above do not include Bremsstrahlung radiation.

CONTAMINATION

Contamination skin dose (mSv.h^{-1})

Uniform deposit (1kBq.cm^{-2})	1.89E+0
0.05 ml droplet (1 kBq)	1.33E+0



Detection

Recommended probes*	
Alpha	
Beta	+ +
Gamma	
X rays	

Derived limits (Bq.cm^{-2})

Removable contamination	
5E+1	
Fixed contamination	
3E+2	

* If no probes are indicated the recommended technique is to use a wipe test in association with a probe or liquid scintillation technique

SHIELDING (mm)

Betas and electrons (Total absorption)

Glass	3.4
Plastic	6.3

Gamma and X rays (half and tenth value thickness)

	½	1/10
Lead	-	-
Steel	-	-

INTERNAL EXPOSURE FOR WORKERS

COMMITTED EFFECTIVE DOSE PER UNIT INTAKE (Sv.Bq^{-1})

Ingestion	f_1	
All compounds	0.800	2.4E-09

Inhalation		1 µm	5 µm
All unspec. compounds	F	8.0E-10	1.1E-09
Some phosphat. det. by comb. cation	M	3.2E-09	2.9E-09
	S		

Highest dose organ: Lungs — **20 mSv ALI$_{ingestion}$**: 8.3E+06 (Bq) — **20 mSv ALI$_{inhalation}$**: 6.3E+06 (Bq)

MAXIMUM RECOMMENDED ACTIVITIES IN LOW LEVEL OR INTERMEDIATE LEVEL LABORATORIES (Bq)

PHYSICOCHEMICAL STATE	*Subject to external exposure requirements which may be more restrictive*					
	Volatility factor (k)	Supervised area		Controlled area		
		Bench	Fume hood	Bench	Fume hood	Glove box
All compounds	0.01	5E+05	5E+06	2E+06	2E+07	2E+09

ALMOST ALL WORK WILL BE CARRIED OUT EITHER BEHIND A PERSPEX SHIELD OR USING PERSPEX TUBE RACKS SO THE DOSE SHOULD BE LIMITED TO FINGERS; WHOLE BODY DOSE WILL BE INSIGNIFICANT.

Handling stock pot Maximum activity 9.25MBq

Handling working stock solution Maximum activity of 3.7MBq

Handling 24 Eppendorf tubes per assay Maximum activity of 0.0185MBq per tube

Handling ^{32}P-spotted P81 paper Maximum activity of 0.0148MBq per paper

	For an activity of 1MBq, from Delacroix					
	High dose	**Syringe**		**Low dose**	**Point source (30cm)**	
	H	23.9	mSv/hr	L	0.12	mSv/hr
		0.398333333	mSv/min		0.002	mSv/min
		0.006638889	mSv/sec		3.33333E-05	mSv/sec
	dose rate	*manipulation*	*manipulated*			
	enter H or L	*time (s)*	*amt (MBq)*	*dose (mSv)*		
Stock pot manipulation	H	5	9.25	0.3070		
Pipetting from working stock into assay tubes	L	60	3.7	0.0074		
Closing lids and transfer to centrifuge	H	60	0.0185	0.0074		
Centrifuge to Vibrax	H	30	0.0185	0.0037		
Vibrax to centrifuge	H	30	0.0185	0.0037		
Remove supernatant and dispose of tube	H	120	0.0185	0.0147		
P81 paper washes	L	120	0.0148	0.0001		
			Total	0.3440	Total dose in mSev	

AMPK SAMS kinase assay - buffers

Homogenisation buffer (sucrose-based) – made up to 1L

- 50mM Tris base (MW 121.1) — 6.055g/L (we want a pH of 8.4 at 4°C)
- 0.25M sucrose (MW 342.3) — 85.6g/L
- 1mM EDTA (from 0.5M stock) — Use 2mL in 1 L (or 0.4533g/L)
- 5mM Na pyrophosphate (MW 446.1) — 2.23g/L
- 50mM NaF (MW 41.98) — 2.1g/L Toxic! Mask/gloves/fumehood!

- **DTT** — 1mM
- **PMSF** — 0.1mM
- **Benzamidine** — 1mM

Added last/to an aliquot of buffer itself
Add 10 µl fresh aliquot of 1M DTT to a 10mL aliquot buffer

- pH the Tris at RT to 7.5!!!!!!!!!!!

Add dH_2O to make up to total volume of 1000mLs

HEPES buffer A (HBA)

- 50mM HEPES (MW 238.3) pH7.5 at 4^{0}C — 11.915 g
- 50mM NaF (MW 41.99) — 2.0995 g
- 5mM NaPP(sodium pyrophosphate) (MW 446.1) — 2.2305 g
- 1mM EDTA (MW 452.2) — 0.4522 g
- 10% (v/v) glycerol — 100mL in 1L
- 1mM DTT
- 4µg/ml trypsin inhibitor
- 0.1mM PMSF (from0.1M stock in ethanol)
- 157µg/ml benzamidine

_____ = **(added to buffer just before use)**

Add dH_2O to make up to total volume of 1000mLs

HGE buffer (HGE) – (i.e. same as HBA omitting the phosphatase inhibitors,NaPP and NaF)

- 50mM HEPES (MW 238.3) pH7.5 at 4^{0}C — 11.915 g
- 1mM EDTA (MW 452.2) — 0.4522g
- 10% (v/v) glycerol — 100mL (in 1 L)
- 1mM DTT
- 4µg/ml trypsin inhibitor
- 0.1mM PMSF (from0.1M stock in ethanol)
- 157µg/ml benzamidine

Make up to 1L with dH_2O

_____ = **(added to buffer just before use)**

100mM ATP stock

dissolve sufficient ATP in 1ml of water to make a 100mM stock.

Check the actual conc by OD260. Dilute a small amount to 1mM then further dilute by 1/100 and read the OD should be 0.15. If it is not correct the label on the stock so it reads accurately.

100mM AMP stock

Make up as for ATP

AMP & SAMS (SAMS = HMRSAMSGLVLKRR)

1mM AMP diluted from 100mM stock in HGE

1mM SAMS diluted from 10mM stock in HGE

SAMS assay mixes

SAMS + AMP

- 1mM SAMS – 200uL
- 1mM AMP – 200uL
- HGE/1% Tx-100 – 200uL

SAMS - AMP

- 1mM SAMS – 200uL
- dH_2O – 200uL
- HGE/1% Tx-100 – 200uL

(N.B. phosphatase inhibitors tend to inhibit AMPK activity slightly)

^{32}P-ATP/$MgCl_2$ 

- γ ^{32}P-ATP * 10µl (can use up to 20µl)
- 100mM ATP (cold) 10µl
- 1M $MgCl_2$ 25µl
- Make up to 1ml with dH_2O 955µl (or 945 µl – see above)

*Hartmann Analytical product code SRP501/100 for 3.7MBq/100µCi in a volume of 10µl

10µl of fresh γ ^{32}P-ATP diluted to 1ml gives 3.7Mbq/ml. Using 5µl of this stock solution per sample equates to 0.0185MBq/sample.

AMPK kinase assay - homogenisation

It is important to keep everything cold until assaying

Sample Homogenisation

WORK QUICKLY ON ICE THROUGHOUT!!

- Prepare 10mL aliquot Homogenisation buffer with fresh PIs (DTT, PMSF, benzamidine)
- Weigh out frozen heart/tissue (100mg tissue ideal) – try to use same amount for each
- Add exactly wt x 5 vol (e.g. 500uL for a 100mg heart) homogenisation buffer
- Mince tissue as best as can with scalpel/fine scissors
- **Homogenise on ice in cryovial tubes**
- Add 10% triton to make final vol of 1% triton (e.g. for 100 µL sample, add 10 µL of 10% Triton X-100)
- Leave to stand for 10 mins
- Cold c'fuge 14,000 rpm 10 mins at 4 ^{o}C
- Transfer soluble top fraction to labelled new 1.6mL epps (can store O/N at 4°C)

BCA assay on homogenates (NB performed on samples diluted in same mannitol or sucrose-based buffer, e.g. 1 in 100 dilution)

- Calculate conc of the non-diluted original samples
- Then dilute these to a set conc with the same mannitol-based buffer (with 1% triton added to the buffer now) in small Falcon – e.g. all to 5mg/mL, then aliquot these out into new labelled epps, e.g. 500 µL in each epp (for liver)
- N.B. for heart, made vol up to ~1.5mL only & aliquoted out into 250 µL aliquots

Preparation of 50:50 protein G-sepharose slurry

- Take protein G-sepharose from freezer (1mL), (?? e.g. P3296-1ML from Sigma)
- Wash the protein G-sepharose beads 3-5 x in PBS (?or IP buffer)
- I.e. add 500uL PBS, C'fuge 14,000 x g for 30 sec, Discard s'natant
- Rpt 500 µL PBS or so; Spin again & discard s'natant
- Finally, resuspend at a ~ 50% slurry (e.g. 500-600 µL PBS)

AMPK kinase assay – Ab preparation & IP

Pre-binding of Antibody

- Set up labelled epps, include duplicates for each sample to allow IPs with & without AMP & a couple of controls
- Add 15 µL of above 50% protein G slurry to a fresh epp (vortex/or pipette up/down prior to aspirating the slurry to ensure beads well-mixed)

- Add 250 µL ice-cold PBS to all epps
- Then add 10uL of sheep γ2-specific Ab to each epp
- **Shaker (vigorous) in COLD ROOM for 2 hours**

Washing of pre-bound Ab/resin

- Remove epps from cold room & spin down *gently* (Nav uses as low as 1,000 x g for 30-60 sec)
- Aspirate s'natant carefully (use cell culture type aspirator with long tips), as much as possible without disturbing matrix
    - WASH 1 – Ice cold PBS/1% triton (add between 500-1000 µL)
    - shake well, spin & aspirate off
    - WASH 2 – Ice cold plain PBS added, low-speed spin
    - Aspirate s'natant off
    - Can store protein G-sepharose adsorbed γ2 Ab in cold room overnight (NOT on shaker!)

Immunoprecipitation

- From Hepes buffer A stock solution take 10mL into small Falcon, add 1mL of stock 10% triton to make 1% triton
- Then add fresh protease inhibitor aliquots from freezer:
    - DTT, PMSF, Benzamidine (10 µL of each per 10mL)
- With new tissue, can use a range of loading protein concentrations (e.g. 250ug, 500ug, 750ug, 1000ug, 1500ug) & place in a volume of hepes buffer A/1% triton (e.g. 500-750uL)
- So **for each sample:**
    - 1) **15-20 µL protein G slurry/adsorbed Ab**
    - 2) **requisite amount of sample** for that conc (e.g. ranging from250 - 1500 µg of protein)
    - 3) **requisite amount of HBA/1% Tx**/protease inhibitors to make total vol of 500 µL

- **Set up all IPs (+ controls) ON SHAKER in COLD ROOM/4°C for 2 hours (Nic uses 1.5-3hrs)**

NB Remember to set up a **'No Ab control'** (to assess background) for each loading sample concentration, e.g. 15-20 µL protein G-sepharose + 250-1500 µg protein + requisite vol hepes buffer A/1% triton (NO Ab in any of these!)

Post-IP washes

- Remove IPs from cold room & aspirate off most of s'natant
- **Start 3 washes – all ICE COLD:**
    - **WASH 1 – Add 1mL Hepes buffer A/1% triton** (Ξ HBA/1% triton)
    - Then low-speed spin ~ 2,000 x g for 30-60 sec
    - Aspirate off s'natant (use tissue culture type aspirator with long tips), leaving matrix at bottom
    - **WASH 2 – HBA alone**
    - **WASH 3 – PBS alone (ICE COLD!!)**
    - After final wash – aspirate off remaining supernatant (ideally using a fine Hamilton microsyringe)

AMPK kinase assay – hot ATP work

SAMS + ATP^{32} → **AMPK ± AMP** → **SAMS-$^{32}PO_4^-$ + ADP**

SAMS assay

- Prepare SAMS + AMP and SAMS – AMP stock mixes (?concs are final concs)
  - 1mM SAMS – 200 µL
  - 1mM AMP – 200 µL (or dH_2O for 'SAMS – AMP')
  - HGE/1% Tx-100 – 200 µL

- **Now prepare SAMS reaction – add the following 25 µL <u>to each pellet</u>:**
  - SAMS assay mix 15 µL (-AMP/+AMP as appropriate)
  - HBA/1% Tx 5 µL (at 50mM)
  - ^{32}P-ATP/$MgCl_2$ 5 µL (added last in hot room only)

- This is a timed reaction, so can either 'spot' 15 µL of the appropriate SAMS assay mix on inner aspect of epp lid and keep lids open until T/F to hot room or potentially add this direct in the hot room. (Can add HEPES direct to pellet first)

Hot room work – controls!

- **Full PPE** – fastened lab coat, two pairs of gloves, eye protection
- **Work behind Perspex screen** & use spill tray
- **Reduce dosage by control of distance, time & shielding** - work quickly
- **Monitor gloves, work area, disposal sink & hybridisation oven for contamination** before, during and after each experiment

Initiation of SAMS reaction

- **Add 5 µL of ^{32}P-ATP/$MgCl_2$ to side of each tube behind Perspex screen**, close all lids rapidly
- Start reaction by brief centrifugation - **high speed for ~20 sec**
- **Immediately place on Vibrax at 37°C (**within incubator chamber/warmer)
- **Time reaction to 30 minutes**

Post-SAMS reaction incubation

- **Immed remove tubes** from Vibrax
- **Briefly centrifuge contents** of tube down again
- **Remove 20 µL of the supernatant** (without disturbing the pellet)
- **Spot aliquot of radioactive mixture onto a P81 paper square** (numbered in pencil beforehand)
- Let square dry for 2-10 seconds
- **Then drop into container of 1% (v/v) orthophosphoric acid using forceps - stir**
- Stir paper, then pour acid away down sink
- **Follow with a water wash**
- **Then repeat acid wash until all free isotope is removed down Perform two washes (5 mins) in phosphoric acid** (i.e. wash several times until the blanks no longer register significantly on the Geiger counter)
- Dry filter paper on a paper towel behind Perspex screen
- **Immerse filter paper in a counting vial containing scintillation fluid** (~3mL/tube Ecoscint from National Diagnostics Cat no. LS-273) & collect counts

NB if immune complex is to be run on a gel, wash pellets free of ^{32}P with several washes in Hepes buffer/1% Triton X100

Then add sample buffer and boil to load on gel

Specific radioactivity of hot ATP

- Assay mix contains 1mM ATP & we use 5 µL/assay
- Count activity of 1 µL ^{32}P-ATP/$MgCl_2$ (spotted onto P81 paper as above)
- If 1µl gives 150,000cpm therefore 5µl gives 750,000cpm

Disposal Methods of Radioactive Material

Radiation User:	Dr Arash Yavari	**RPS:**	Dr James Brown
Group:	Cardiovascular Medicine/Watkins	**Date of Assessment:**	24th Jan 2012

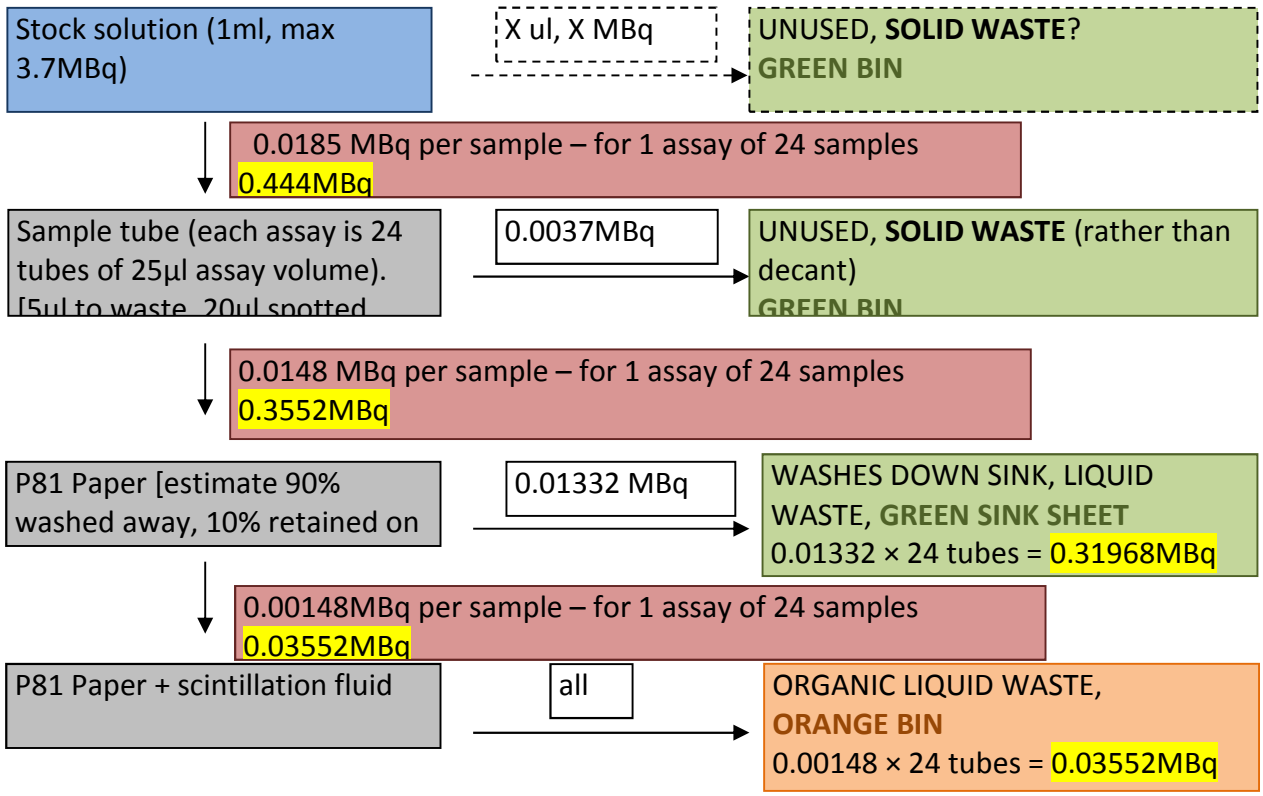
Overview of Project to be undertaken:

AMPK assays: determining the activity of AMP Kinases by measuring the rate of incorporation (phosphorylation) of γ-^{32}P ATP into synthetic peptides

Radionuclide to be used (MBq): γ-^{32}P ATP

Amount of Radionuclide to be used per Experiment: 0.444MBq

*For each stage of the experiment - Detail the amount of radionuclide (MBq) that you **estimate** to be taken up or lost to waste. Detail for each stage whether this waste is liquid or solid.*



Estimated Total amount of Liquid Waste: 0.32MBq	**Estimated Total amount of Solid Waste:** 0.0888 MBq	**Estimated Total amount of Organic Liquid Waste:** 0.03552 MBq
Est. Liquid Waste as a % of the Total used: 72%	**Est. Solid Waste as a % of the Total Used:** 20%	**Est. Organic Liquid Waste as a % of the Total Used:** 8%

Determine if the estimated waste figure calculated above is a true result. Affix any actual data gathered to this document to support the Waste Management Protocol.
See affixed data from 24th August 2011.

Actual Liquid Waste as a % of the Total Used: 79.4%	**Actual Solid Waste as a % of the Total Used:** 20%	**Actual Organic Liquid Waste as a % of the Total Used:** 0.6%

Revision to waste management protocol for AMPK kinase assay using data from an actual sample run (24/08/2011, raw data in Dr A. Yavari's Lab Book)

Unused solid waste – (GREEN BIN SHEET)

- 5 µL of each 25 µL reaction mix (ie 20%) is always disposed of in the reaction tube as solid waste
- Other solid waste (pipette tips and paper towel) is not significant compared to the reaction waste and can be considered within the solid waste described above.
- Thus for every experiment, **solid waste** effectively comprises **20%** of the total waste.

The remaining 80% of waste is split between 'liquid' and 'organic liquid' as follows:

Organic liquid waste (ORANGE BIN)

- Mean sample count in the run = 7055 cpm
- For a reaction run of 24 samples this equates to **169,320 cpm**

Liquid waste - washes down sink (GREEN SINK SHEET)

- cpm of ['hot' orthophosphoric acid – plain orthosphosphoric acid] measured as 544.5 cpm
- This is for 20µL of orthophosphoric acid spotted onto P81 paper, so for the total 900mL actually used (i.e. 3 x 300mL beakers), this gives a total of **2.45×10^7 cpm**

Thus 80% of all waste is 2.46×10^7 cpm [169,320 cpm + 2.45×10^7 cpm].

Proportions of waste are:

- Solid waste 20%
- Organic liquid waste 0.6%
- Liquid waste 79.4%

For an assay of 24 samples using 0.444MBq γ-^{32}P ATP:

- Solid waste 0.0888 MBq
- Organic liquid waste 0.00266 MBq
- Liquid waste 0.35256 MBq

These figures have been entered in to the waste stream table and will be used for all future assays.

Dr Arash Yavari, January 2012

University of Oxford COSHH Assessment Form

Date: 21.8.17

Department: **Cardiovascular Medicine** *Location of work*: **WTCHG, Lab 3N**	*Persons involved*: **Arash Yavari, Sahar Ghaffari**

Description of procedure:

Immunoprecipitation and AMP kinase assay using ^{32}P – Use of Protease & Phosphatase Inhibitors

N.B. A separate radiation risk assessment covers radiation part of this work

Substances used	*Quantities used*	*Frequency of use*	*Hazards identified*	*Exposure route*
Dithiothreitol (DTT)	Low mM concentrations used only	Fortnightly - monthly	 H302 Harmful if swallowed. H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation	Contact with skin Inhalation theoretical but unlikely
Phenylmethanesulfonyl fluoride (PMSF)			 H301 Toxic if swallowed. H314 Causes severe skin burns and eye damage.	
Benzamidine			 H302 Harmful if swallowed. H319 Causes serious eye irritation.	
Sodium fluoride			 H301 Toxic if swallowed. H315 Causes skin irritation. H319 Causes serious eye irritation.	
Sodium pyrophosphate			 H315 Causes skin irritation. H319 Causes serious eye irritation. H335 May cause respiratory irritation.	
1% (v/v) orthophosphoric acid (made from 85% stock sol^{n})	~1l	Fortnightly-monthly	 H318 Causes serious eye damage H302 Harmful if swallowed H314 Causes severe skin burns and eye damage	Contact with skin

Could a less hazardous substance (or form of the substance) be used instead? **No**

Justify not using it:

Gold standard assay for assessing functional activity of AMPK by initially immunoprecipitating the kinase with AMPK subunit-specific antibodies, then assessing its ability to incorporate radiolabelled phosphate into a synthetic substrate

Davies SP, Carling D, Hardie DG (1989) Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic-AMP-dependent protein kinase, studied using a specific and sensitive peptide assay. Eur J Biochem 186: 123–128

What measures have you taken to control risk?

***Engineering controls*:**
Protease inhibitors - Preparation of small aqueous aliquots wherever possible and storage to reduce need for repeated preparation. Use of fume hood when preparing stocks of sodium fluoride aliquots. Orthophosphoric acid working solution (1%) made up in fume hood. Refer to COSHH Assessment for Safe Use of Acids and Alkalis.

***PPE*:**
Laboratory coat, gloves, eye protection used at all times during experiment. Good laboratory practice & hand washing

Management measures:
Correctly label all hazardous solutions.

Checks on control measures:
Users must check that chemical fume hood gauges indicate satisfactory air flow and that sash window is used at an appropriate working height. If air flow failures are indicated stop work & make safe, and report the problem to Lab Support
Annual inspection and validation of MSCs and chemical fume hoods are carried out to monitor performance of LEV; inspection reports are available from WTCHG H&S Officer.

Is health surveillance required? No	*Training requirements*: Ensure new personnel are aware of hazards

***Emergency procedures*:**	***Waste disposal*:**
Inhalation: Move person into fresh air. If not breathing, give artificial respiration. Consult a physician. *Skin contact:* Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. *Eye contact:* Flush eyes thoroughly with water for 15 min. *Swallowing:* Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. In all cases of exposure, consult a doctor.	Standard waste disposal of dilute solutions to drains Radioactive waste – see specific radiation risk assessment

Name and position of assessor: Dr Arash Yavari / Dr James Brown Signature: